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Characterization of Two cDNAs Encoding Small GTP-Binding Proteins (Accession Nos. AF165095 and AF165096) from Immature Cotton (*Gossypium hirsutum* L.) Locules

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Small monomeric GTP-binding proteins (G-proteins) are important signal transducers found in all eukaryotic organisms. Conformational changes in protein structure occur when G-proteins are cycled between an active state when GTP is bound and an inactive state after GTP hydrolysis. Classification of members of the G-protein family are based upon sequence homology or divergence within regions for nucleotide binding and hydrolysis. In plants, the Ras superfamily is sub-divided into the Ras, Rab, Arf/Sar, Rho/Rac and Ran families (Bischoff et al., 1999).

Two cDNAs (GhRab11a and GhRab11b) encoding small G-proteins were obtained from developing cotton locules using RT PCR and 5' RACE PCR. Homology searches using BLASTP showed that the cotton G-proteins were homologous to Rab G-proteins from other plants but had little homology to the cotton Rab G-protein isolated from cotyledons (Jenkins et al., 1999). The polypeptide sequences of GhRab11a and GhRab11b have the most homology to Rab11J from *Lotus japonicus* root nodules (Borg et al., 1997). Although *L. japonicus* Rab11J was originally isolated from root nodule tissue, greater expression of Rab11J was associated with aerial parts of the plant than with root nodules (Borg et al., 1997).

In general, the Rab family of G-proteins functions to regulate the specificity and directionality of vesicular transport from a source to a target compartment. The Rab11 subfamily is mainly involved in transporting secretory vesicles from the trans-Golgi network to the plasma membrane (Bischoff et al., 1999). In mammals, Rab11 co-segregates with H+/K+-ATPase and is involved in the apical targeting of vesicles in epithelial cells throughout the gastrointestinal tract (Calhoun and Goldenring, 1997). In transgenic tobacco plants, over-expression of OsRab11a led to a reduction in apical dominance, elevated levels of cytokinin and increased resistance to viral infection (Sano et al., 1994). In pea plants, expression of PsRab11b was localized to rapidly expanding cells that have highly active Golgi (Nagano et al., 1995). A Rab 11-like gene discovered in ripening mangos was found to be involved in trafficking cell wall modified enzymes through the trans-Golgi network (Zainal et al., 1996).

At six days post-anthesis, cotton fiber cells are elongating at nearly their optimal rate. Cell expansion in all parts of the developing seed is also very active at this stage of development. Due to the rapid growth rate of fiber and seed, biogenesis and deposition of large quantities of non-cellulosic cell wall polymers for expanding primary cell

walls are necessary. We propose that GhRab11a and GhRab11b are involved in coordinating the transport of vesicles containing cell wall polymers and enzymes from Golgi to the plasma membrane or vacuolar membrane and play a central role in plant cell expansion.

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TABLE 1.Characterization of GhRab11a and GhRab11b from Immature Cotton Locules

Organism:

Upland cotton, *Gossypium hirsutum* L., Texas Marker-1

Source:

PolyA+ RNA from 6 days post anthesis (DPA) cotton locules was reverse transcribed and PCR amplified using primers that were designed from conserved sequences in the Rab family of small G-proteins. Full-length cDNA clones were obtained using 5' Rapid Amplification of cDNA Ends (5' RACE). Amplified products were cloned in the plasmid PCRII (Invitrogen).

Sequencing:

Both strands of each plasmid were sequenced using the 4000 L Automated DNA Sequencer (LI-COR) with the cycle sequencing protocol from the SequiTherm EXCEL II Long-Read Sequencing Kit-LC (Epicentre Technologies).

Features of the cDNA:

The cDNA encoding GhRab11a is 672 bp in length and contains a 5' UTR (186 bp) and a 3' UTR (49 bp). The cDNA encoding GhRab11b is 672 bp in length and contains a 5' UTR (237 bp) and a 3' UTR (49 bp). The 5' UTR's of GhRab11a and GhRab11b are not homologous and diverge by 33%.

Features of the predicted amino acid sequence:

The open reading frame of GhRab11a and GhRab11b consists of 224 amino acids, which are 98.7% identical to each other. A motif search using ScanProsite revealed a cAMP Kinase phosphorylation site (position 98-101), protein kinase C phosphorylation sites (positions 53- 55, 114-117, 138-141, and 157-160) and a GTP-binding site (position 157-160).